Am. J. Trop. Med. Hyg., 88(5), 2013, pp. 850–854
doi:10.4269/ajtmh.12-0671

Efficacy of Chloroquine for the Treatment of Uncomplicated *Plasmodium falciparum* Malaria in Honduras

Rosa Elena Mejia Torres,* Engels Ilich Banegas, Meisy Mendoza, Cesar Diaz, Sandra Tamara Mancero Bucheli, Gustavo A. Fontecha, Md Tauqeer Alam, Ira Goldman, Venkatachalam Udhayakumar, and Jose Orlinder Nicolas Zambrano

National Malaria Laboratory, National Department of Surveillance, Ministry of Health, Honduras; National Malaria Eradication Service (SNEM), Ministry of Public Health, Ecuador; Area of Health Surveillance and Disease Control, Pan American Health Organization, Honduras; Department of Masters in Infectious diseases and Zoonoses (MEIZ)-Microbiology School, National Autonomous University of Honduras (UNAH), Tegucigalpa, Honduras; Atlanta Research and Education Foundation, Decatur, Georgia; Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia; National Malaria Program, Department of Health Promotion, Ministry of Health, Honduras

Abstract. Chloroquine (CQ) is officially used for the primary treatment of *Plasmodium falciparum* malaria in Honduras. In this study, the therapeutic efficacy of CQ for the treatment of uncomplicated *P. falciparum* malaria in the municipality of Puerto Lempira, Gracias a Dios, Honduras was evaluated using the Pan American Health Organization—World Health Organization protocol with a follow-up of 28 days. Sixty-eight patients from 6 months to 60 years of age microscopically diagnosed with uncomplicated *P. falciparum* malaria were included in the final analysis. All patients who were treated with CQ (25 mg/kg over 3 days) cleared parasitemia by day 3 and acquired no new *P. falciparum* infection within 28 days of follow-up. All the parasite samples sequenced for CQ resistance mutations (*pfcrt*) showed only the CQ-sensitive genotype (CVMNK). This finding shows that CQ remains highly efficacious for the treatment of uncomplicated *P. falciparum* malaria in Gracias a Dios, Honduras.

INTRODUCTION

Development of resistance to chloroquine (CQ) and other drug treatment in *Plasmodium falciparum* malaria has led the World Health Organization (WHO) to change its recommendations to adopt artemisinin combination therapy (ACT) as the first-line drug for the treatment of *P. falciparum* malaria cases in most endemic countries. In the Americas, CQ-resistant *P. falciparum* has been documented in all South American countries endemic for malaria since the 1950s,[1] including Brazil,[2] Bolivia, Colombia,[3] Ecuador, Peru,[4] Guyana,[5] Suriname,[6] and Venezuela.[7,8] All of these countries have changed their drug policies in alignment with WHO recommendations to adopt ACT.[9]

In contrast, for Central American countries (except Panama) and the island of Hispaniola, CQ continues to be the first line of treatment of *P. falciparum* malaria, because there is no evidence for the presence of CQ resistance in Central America outside of Panama, Mexico, or the Caribbean islands.[10] Panama is the only Central American country with documented CQ-resistant *P. falciparum* malaria[11] and as a result, changed its first line of treatment to sulfadoxine-pyrimethamine (SP) combination in 2004.

Honduras has the highest malaria burden in Central America.[12] Honduras accounted for 69%, 84%, and 91% of all *P. falciparum* cases reported in Central America in 2007, 2008, and 2009, respectively.[13] A remarkable decrease in the transmission of the disease has been observed in the country in the last decade: from 35,125 cases in 2000 to 8,368 cases by 2008.[13] Most of the malaria cases are found in the northeastern region of the country, particularly the state of Gracias a Dios, which reported 33% (2,798 cases) of all malaria cases in 2008 and accounted for 69–94% of *P. falciparum* cases over 2007–2009.[14]

In Honduras, CQ efficacy was determined using *in vivo* protocol only in 1981, and since that time, no additional studies have been conducted.[15] To confirm the efficacy of CQ for the treatment of *P. falciparum* malaria in Honduras, an *in vivo* study was conducted using Pan American Health Organization (PAHO) – WHO protocol with a 28-day follow-up. In addition, *P. falciparum* isolates collected from this study were tested for the presence of CQ-associated molecular markers in the *pfcrt* gene.

MATERIALS AND METHODS

Study location and patient enrolment. The study was carried out from September of 2008 to September of 2009 in the municipality of Puerto Lempira, Gracias a Dios, Honduras (Figure 1), a highly endemic area for *P. falciparum* malaria. This study was approved by the institutional ethical review committee of Ethics Committee of the Medical Sciences Faculty of the National Autonomous University of Honduras (UNAH-IRB 00003070). All participants provided written consent to participate in the study. Patients residing within a radius of 30–45 minutes of travel time (car or boat) were included, because this area was convenient for the study team to conduct follow-up observations. Criteria for patient inclusion were uncomplicated *P. falciparum* malaria as described by national and international definitions.[10] This criteria included participants aged between 6 months and 60 years with microscopically detectable mono-infection with *P. falciparum*, parasitemia levels greater than 250 but less than 50,000 asexual forms/μL, willingness to attend follow-up visits for 28 days, and willingness to participate in the study and provide informed written consent. The exclusion criteria included pregnancy or lactation (pregnancy test was performed for women of childbearing age), signs of severe malaria,[16] underlying chronic disease (cardiac, renal, or hepatic disease or malnutrition), history of allergy to CQ, and presence of mixed infection with different species of malaria parasites.

Sample size. The sample size was determined according to the proportion of treatment failures expected in this population. Assuming a CQ treatment failure rate of 5% in a population of infinite size, a power of 80%, and a significance level of 5%, 42 patients per treatment would be required. If allowing for 15% attrition, 49 patients would be needed. A total of 69 patients who were determined to have only *P. falciparum* infection based

*Address correspondence to Rosa Elena Mejia Torres, National Malaria Laboratory, National Department of Surveillance, Ministry of Health, Honduras. E-mail: rosaelenamejiatorres@yahoo.com

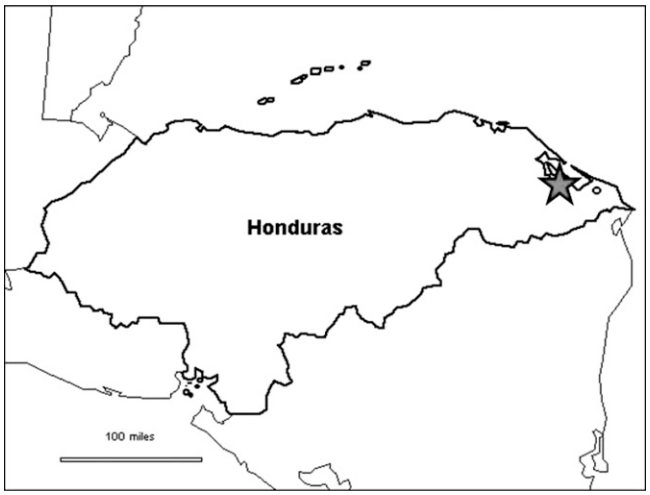

FIGURE 1. Map of Honduras showing the study site for *in vivo* drug efficacy study in Puerto Lempira, Honduras.

on microscopic diagnosis were enrolled for this study, and 68 of the patients completed the follow-up until day 28.

Microscopy and sample collection. Blood smears were stained with 3% Giemsa for 30 minutes at room temperature. Smears were analyzed by experienced microscopists. The parasite density was determined by using the PAHO–WHO criteria assuming 6,000 leukocytes/μL. A total of 137 (as one failed to complete follow-up) blood spots (Whatman number 3), including 69 samples collected at the time of enrolment and 68 samples collected at the 28-day follow-up, was dried and stored at –20°C until use for molecular analysis.

Drug treatment and follow-up. The antimalarial drug and treatment schedule used was CQ at 25 mg/kg body weight divided into daily doses over 3 days: 10 mg/kg on day 1, 10 mg/kg on day 2, and 5 mg/kg on day 3. SP and quinine were chosen as alternative drugs if CQ was not effective. Supervised treatment was administered to all patients followed by clinical and parasitological evaluation on days 0, 1, 2, 3, 7, 14, 21, and 28. Therapeutic response was classified in patients who completed the follow-up using the definitions described in the standard PAHO–WHO protocol.[17]

To facilitate the administration of proper dosage, tables of weight-based dosing were available. Treatment choice and dosage were administered by the same team under the supervision of clinical personnel. Patients were monitored for 30 minutes after drug administration for adverse reactions or intolerance. Paracetamol was administered for fever (axillary temperature higher than 38.5°C) with doses of 10 mg/kg (maximum = 500 mg) four times per day conditional on the presence of fever.

Patient follow-up. Patients enrolled before treatment with CQ received supervised treatment in the Health Center for follow-up until day 28. Because only patients with uncomplicated malaria treated with drugs with established safety profiles were included in the study, daily monitoring was not required. Both legal guardians (parents of children under 18 years) and patients were informed about the significance of treatment and the importance of the 28-day follow-up.

Molecular diagnosis and genotyping for CQ resistance markers. Genomic DNA from *P. falciparum*-positive filter paper blood spots was isolated using the Tris–ethylenediaminetetraacetic acid (EDTA) method as described earlier.[18] To confirm the parasite species present in the samples, an 18s rRNA nested polymerase chain reaction (PCR) was performed with primers and cycling conditions as described by Singh and others.[19] PCR results confirmed presence of *P. falciparum* infection in 68 patients, and 1 patient was found to have *P. vivax* but not *P. falciparum* infection as reported recently.[20]

To determine if there is any parasite with CQ-resistant genotype, the DNA was subjected to the PCR amplification of the partial regions of *pfcrt* (covering codons 72–76) using a nested PCR approach. The primary PCR was performed with 5′-AGCAAAAATGACGAGCGTTATAG-3′ (F) and 5′-ATTGGTAGGTGGAATAGATTCTC-3′ (R) primers with the following cycling parameters: initial denaturation at 94°C for 10 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 30 seconds followed by a final extension at 72°C for 10 minutes.[21] Secondary PCR was done using 5′-TTTTTCCC-TTGTCGACCTTAAC-3′ (F) and 5′-AGGAATAAACAA-TAAAGAACATAATCATAC-3′ (R) primers. The cycling parameters for secondary PCR were the same as for the primary PCR, except that annealing was set at 56°C for 30 seconds and the number of cycles was reduced to 30. Sequencing of gene fragments was carried out on both strands with their respective nested primers using the standard sequencing protocols on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA) as described earlier.[21]

Statistics. Double entry of data was executed and then analyzed using Excel. Median and geometric means were used for analysis of continuous variables and percentages for categorical and nominal variables.

RESULTS

A total of 4,827 febrile patients attended two Health Centers, of whom 3,714 patients were negative for malaria according to light microscopy (Figure 2). These patients were referred to medical consultation to investigate other diseases; 1,113 patients were diagnosed with malaria, of whom 791 (71%) patients had *P. vivax*, 263 (24%) patients had *P. falciparum*, and 59 (5%) patients showed a mixed infection with both species. Of the total cases of *P. falciparum* malaria captured in the sentinel sites, only 69 cases were included in this study (26%): the remainder of cases did not meet protocol inclusion criteria or the patient refused to participate. Among these 69 patients included in the study, 1 patient was excluded from the final analysis, because this patient was found to have only *P. vivax* but not *P. falciparum* infection based on subsequent PCR test.[20] Demographic data of study participants are given in Table 1.

All patients selected for the study were clinically evaluated according to height, weight, physical examination, and blood pressure on day 0, whereas temperature and blood smear examination were done on days 0, 1, 2, 3, 7, 14, 21, and 28 (Figure 3). At the end of evaluations on day 7, all patients were parasite-negative (absence of microscopically detectable asexual stages in blood), and none developed parasitemia through day 28 of the trial, indicating 100% efficacy of CQ (Figure 4). None of the patients reported having taken antimalarial drugs on their own (CQ, primaquine, or SP); 57 patients (95%) took analgesic, antipyretic, and/or anti-inflammatory medications to reduce symptoms and discomfort.

Molecular markers associated with CQ resistance. The *pfcrt* gene sequencing data for 68 samples showed no mutations within amino acid residues 72–76. All the samples had the

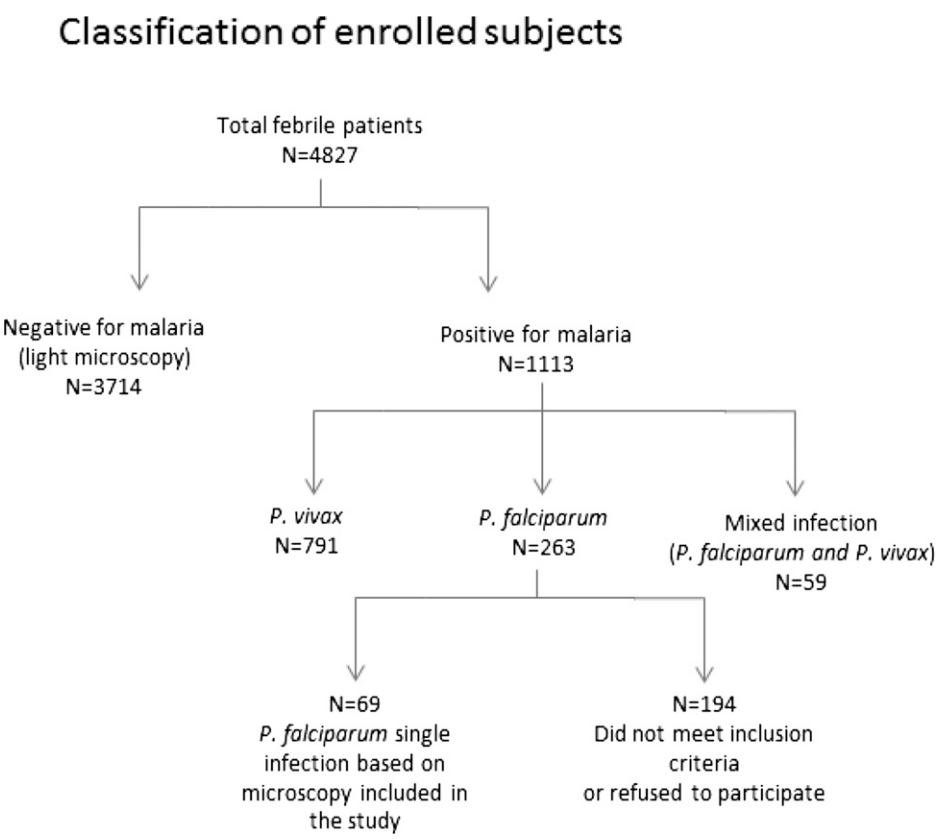

FIGURE 2. Flowchart of the enrolment process for *in vivo* drug efficacy study based on microscopic diagnosis in Puerto Lempira, Honduras.

CVMNK amino acid sequence (CQ-sensitive ancestral genotype) in this region, confirming the absence of parasites with CQ-resistant *pfcrt* allele.

DISCUSSION

The emergence of drug-resistant strains of *P. falciparum* has contributed to worldwide resurgence of malaria in recent decades,[22] and it is associated with increased mortality and morbidity.[23] Drug susceptibility usually relies on various factors, such as the intensity of infection, immune status, plasma concentrations of the drug, and duration of drug application[24–26]; however, the inherent capacity of the parasite to tolerate drugs is mostly based on its genotype.[27,28] We conducted this study to evaluate *in vivo* effectiveness of CQ against *P. falciparum* malaria infections and determine the *pfcrt* genotypes of the *P. falciparum* strains currently circulating in a highly endemic region of Honduras.

Although Honduras has not reported resistance to the current first-line antimalarial drugs CQ and primaquine, it remained unclear if the efficacy of CQ has changed over the time, and there have been occasional local anecdotal reports of treatment failure by both physicians and patients. Another concern was whether any potential misuse of CQ in the local population has altered the clinical efficacy of this drug. Recently, the state of Gracias a Dios has become a major hub for human migration from South America, where CQ resistance is fixed in *P. falciparum* parasites. This finding has

TABLE 1
Characteristics of study participants enrolled in the *in vivo* drug efficacy study in Puerto Lempira, Honduras

Characteristic	*N* = 68*
Median age in years (range)	17 (1–40)
Children under 5 years (%)	7, 10
Children 5–15 years (%)	21, 31
Adults	40, 59
Males (%)	39, 57
Axillary temperature ≥ 37.5°C (day 0; %)	46
Geometric mean parasite density (μL^{-1}; day 0)	6,268

*Although 69 patients were enrolled based on the microscopic diagnosis of *P. falciparum*, 1 patient was excluded from the final analysis, because subsequent PCR experiments determined presence of *P. vivax* infection but not *P. falciparum* infection in that patient.

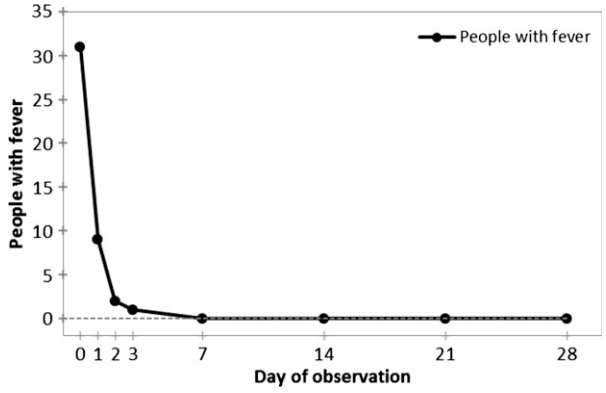

FIGURE 3. Number of study participants with fever (> 37.5°C) by day of observation in the *in vivo* drug efficacy study in Puerto Lempira, Honduras.

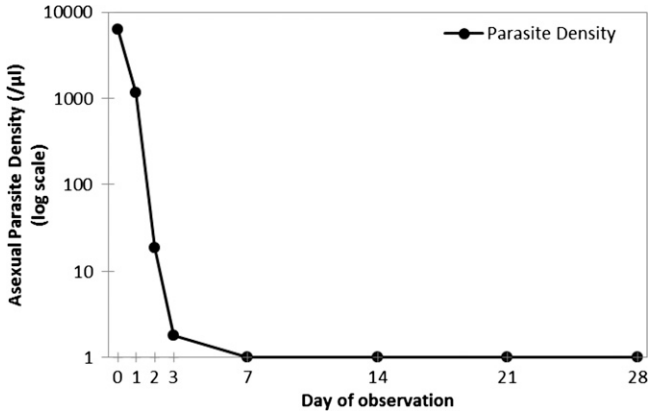

FIGURE 4. Geometric means of asexual parasite density by day of observation in the *in vivo* drug efficacy study in Puerto Lempira, Honduras.

raised concern about potential importation of CQ-resistant parasites through human migrants. Therefore, continuous monitoring for the CQ efficacy and molecular surveillance for CQ-resistant *pfcrt* genotype in this region are essential for the support of current drug policy.

Because there are different parameters of clinical and parasitological assessment to evaluate resistance to antimalarial drugs, standard protocols were used in this study.[29] We used the PAHO–WHO standard methodology and followed patients for 28 days to assess the efficacy of CQ for the treatment of uncomplicated *P. falciparum* infection. In this study, the most important finding is the 100% efficacy of CQ treatment of clearing *P. falciparum* infection during the 28 days of follow-up. This finding is further confirmed by the exclusive presence of only CQ-sensitive ancestral codons of the *pfcrt* gene (CVMNK genotype) in all the parasite samples tested. This study is consistent with the research conducted 3 decades ago,[15] which showed, at the regional level, the usefulness of CQ as a first-line drug. Our study provides strong support for the continuation of CQ for the primary treatment of malaria in Honduras for now.

This study was carried out in the area with the highest incidence of malaria in Honduras and did not include patients from other regions of the country. Although the data from this study cannot be extrapolated to the rest of the country, recent results obtained by Jovel and others[30] confirm that *P. falciparum* isolates from other regions of the country also possessed a susceptible wild-type *pfcrt* genotype. Furthermore, they showed that CQ-resistant alleles of *pfmdr1*, sulphadoxine-resistant *pfdhps* alleles, and pyrimethamine-resistant *pfdhfr* alleles were also not detectable in this region. Because conducting *in vivo* drug efficacy trials can be expensive, it is reasonable to continuously monitor the parasite populations from different parts of the country for the presence of CQ-resistant *pfcrt* allele as an early warning signal for the potential emergence of CQ resistance.

The findings from this study are consistent with recent reports indicating that CQ remains efficacious for the treatment of falciparum malaria in Nicaragua[31] and Haiti.[32] Collectively, these findings are consistent with continuation of CQ as the primary drug of choice for the treatment of malaria in this region. It is still unknown why CQ-resistant parasites have failed to establish in Central American regions outside of Panama, although CQ-resistant parasites are fixed in South America and have been found in Panama.[11] Implementation of an active molecular surveillance program to detect emergence of any CQ-resistant *P. falciparum* parasites will help to develop appropriate measures to prevent the spread of resistant parasites and evaluate the efficacy of CQ for continued treatment of falciparum malaria in this region.

In conclusion, this study showed evidence that there is no resistance to CQ, which is the first-line drug used in the country, according to the National Guidelines for malaria.[10] However, it is necessary to maintain regular monitoring of therapeutic efficacy of antimalarial drugs with standardized methodology and rigorous quality control. An early indicator is the presence of CQ-resistant molecular markers, which can be monitored through the application of routine molecular surveillance of *P. falciparum* isolates in the country.

Received October 31, 2012. Accepted for publication November 30, 2012.

Published online March 4, 2013.

Acknowledgments: The authors thank Efrain Burgos, Director of the Department of Health of Gracias a Dios, and Edgardo L. Barahona, technician in environmental health of the state, for providing support in carrying out this study. Rosalba Quintero Montana trained the microscopists in the study in diagnostics. Prabhjot Singh helped in drafting and revised the final paper. We also thank the members of the Department of Health of Gracias a Dios and the Hospital of Puerto Lempira, where this study was carried out.

Financial support: This work was funded by the US Agency for International Development (USAID) under the USAID Pan American Health Organization agreement for the Amazon Malaria Initiative Network for Surveillance of Anti-Malarial Drug Resistance Project 527A-00-08-00026-00 and the Ministry of Health of Honduras.

Authors' addresses: Rosa Elena Mejia Torres, Engels Ilich Banegas, and Meisy Mendoza, National Malaria Laboratory, National Department of Surveillance, Ministry of Health, Honduras, E-mails: rosaelenamejiatorres@yahoo.com, engelsbanegas@yahoo.com, and elimeis@hotmail.com. Cesar Diaz, National Malaria Eradication Service (SNEM), Ministry of Public Health, Ecuador, E-mail: diazcortezert@gmail.com. Sandra Tamara Mancero Bucheli, Pan American Health Organization, Honduras, E-mail: mancerot@paho.org. Gustavo A. Fontecha, Department of Masters in Infectious diseases and Zoonoses-Microbiology School, National Autonomous University of Honduras (UNAH), Tegucigalpa, Honduras, E-mail: gustavo.fontecha@unah.edu.hn. Md Tauqeer Alam, Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta Research and Education Foundation, Decatur, GA, E-mail: hsf1@cdc.gov. Ira Goldman and Venkatachalam Udhayakumar, Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, E-mails: ifg1@cdc.gov and vxu0@cdc.gov. Jose Orlinder Nicolas Zambrano, National Malaria Program, Department of Health Promotion, Ministry of Health, Honduras, E-mail: jnicolaszambrano@ yahoo.es.

REFERENCES

1. Peters W, 1971. Malaria. Chemoprophylaxis and chemotherapy. *BMJ 2:* 95–98.
2. Ferraroni JJ, Alencar FH, Shrimpton R, 1983. Multiple drug resistance in falciparum malaria from Brazil. *Trans R Soc Trop Med Hyg 77:* 138–139.
3. Comer RD, Young MD, Porter JA Jr, Gauld JR, Merritt W, 1968. Chloroquine resistance in *Plasmodium falciparum* malaria on the Pacific coast of Colombia. *Am J Trop Med Hyg 17:* 795–799.
4. Marquino W, MacArthur JR, Barat LM, Oblitas FE, Arrunategui M, Garavito G, Chafloque ML, Pardave B, Gutierrez S, Arrospide N, Carrillo C, Cabezas C, Ruebush TK 2nd, 2003. Efficacy of chloroquine, sulfadoxine-pyrimethamine, and mefloquine for the treatment of uncomplicated *Plasmodium*

falciparum malaria on the north coast of Peru. *Am J Trop Med Hyg 68:* 120–123.
5. Caraballo A, Rodriguez-Acosta A, 1999. Chemotherapy of malaria and resistance to antimalarial drugs in Guyana area, Venezuela. *Am J Trop Med Hyg 61:* 120–124.
6. Oostburg BF, 1973. Chloroquin-resistant tropical malaria in south Surinam. *Ned Tijdschr Geneeskd 117:* 693–694.
7. Ache A, Escorihuela M, Vivas E, Paez E, Miranda L, Matos A, Perez W, Diaz O, Izarra E, 2002. *In vivo* drug resistance of falciparum malaria in mining areas of Venezuela. *Trop Med Int Health 7:* 737–743.
8. Cortese JF, Caraballo A, Contreras CE, Plowe CV, 2002. Origin and dissemination of *Plasmodium falciparum* drug-resistance mutations in South America. *J Infect Dis 186:* 999–1006.
9. Marquino W, Huilca M, Calampa C, Falconi E, Cabezas C, Naupay R, Ruebush TK 2nd, 2003. Efficacy of mefloquine and a mefloquine-artesunate combination therapy for the treatment of uncomplicated *Plasmodium falciparum* malaria in the Amazon Basin of Peru. *Am J Trop Med Hyg 68:* 608–612.
10. Secretaria de Salud de Honduras, 2010. *Norma de Malaria en Honduras. Secretaria de Salud SdRP, Direccion General de Promocion de la Salud, Programa Nacional de la Prevencion y Control de la Malaria*. Tegucigalpa, Honduras: Secretaria de Salud de Honduras.
11. Samudio F, Santamaria AM, Obaldia N 3rd, Pascale JM, Bayard V, Calzada JE, 2005. Prevalence of *Plasmodium falciparum* mutations associated with antimalarial drug resistance during an epidemic in Kuna Yala, Panama, Central America. *Am J Trop Med Hyg 73:* 839–841.
12. WHO, 2010. *World Malaria Report: Global Malaria Programme*. Geneva: World Health Organization.
13. Pan American Health Organization, 2012. PAHO Health Information Platform-Malaria Surveillance Indicators. Available at: www.paho.org/malariastats. Accessed February 10, 2012
14. National Malaria Programme, 2011. *Situation Report of Malaria in 2011: National Malaria Programme*. Tegucigalpa, Honduras: Ministry of Health.
15. Nguyen-Dinh P, Hobbs JH, Campbell CC, 1981. Assessment of chloroquine sensitivity of *Plasmodium falciparum* in Choluteca, Honduras. *Bull World Health Organ 59:* 641–646.
16. Warsame M, Kimbute O, Machinda Z, Ruddy P, Melkisedick M, Peto T, Ribeiro I, Kitua A, Tomson G, Gomes M, 2007. Recognition, perceptions and treatment practices for severe malaria in rural Tanzania: implications for accessing rectal artesunate as a pre-referral. *PLoS One 2:* e149.
17. World Health Organization/Pan American Health Organization, 1998. *Assessment of Therapeutic Efficacy of Medicaments to Treat Uncomplicated P. falciparum malaria in the Americas. Report No.: PS/HCP/HCT/113/98*. Washington, DC: WHO, OPS.
18. Bereczky S, Martensson A, Gil JP, Farnert A, 2005. Short report: rapid DNA extraction from archive blood spots on filter paper for genotyping of *Plasmodium falciparum*. *Am J Trop Med Hyg 72:* 249–251.
19. Singh B, Bobogare A, Cox-Singh J, Snounou G, Abdullah MS, Rahman HA, 1999. A genus- and species-specific nested polymerase chain reaction malaria detection assay for epidemiologic studies. *Am J Trop Med Hyg 60:* 687–692.
20. Fontecha GA, Mendoza M, Banegas E, Poorak M, De Oliveira AM, Mancero T, Udhayakumar V, Lucchi NW, Mejia RE, 2012. Comparison of molecular tests for the diagnosis of malaria in Honduras. *Malar J 11:* 119.
21. Griffing S, Syphard L, Sridaran S, McCollum AM, Mixson-Hayden T, Vinayak S, Villegas L, Barnwell JW, Escalante AA, Udhayakumar V, 2010. pfmdr1 amplification and fixation of pfcrt chloroquine resistance alleles in *Plasmodium falciparum* in Venezuela. *Antimicrob Agents Chemother 54:* 1572–1579.
22. Butler WP, Roberts DR, 2000. Malaria in the Americas: a model of reemergence. *Mil Med 165:* 897–902.
23. PAHO, 2001. Situation of malaria programs in the Americas. *Epidemiol Bull 22:* 10–14.
24. Le Jouan M, Jullien V, Tetanye E, Tran A, Rey E, Treluyer JM, Tod M, Pons G, 2005. Quinine pharmacokinetics and pharmacodynamics in children with malaria caused by *Plasmodium falciparum*. *Antimicrob Agents Chemother 49:* 3658–3662.
25. Francis D, Nsobya SL, Talisuna A, Yeka A, Kamya MR, Machekano R, Dokomajilar C, Rosenthal PJ, Dorsey G, 2006. Geographic differences in antimalarial drug efficacy in Uganda are explained by differences in endemicity and not by known molecular markers of drug resistance. *J Infect Dis 193:* 978–986.
26. Aubouy A, Bakary M, Keundjian A, Mbomat B, Makita JR, Migot-Nabias F, Cot M, Le Bras J, Deloron P, 2003. Combination of drug level measurement and parasite genotyping data for improved assessment of amodiaquine and sulfadoxine-pyrimethamine efficacies in treating *Plasmodium falciparum* malaria in Gabonese children. *Antimicrob Agents Chemother 47:* 231–237.
27. Andriantsoanirina V, Ratsimbasoa A, Bouchier C, Tichit M, Jahevitra M, Rabearimanana S, Raherinjafy R, Mercereau-Puijalon O, Durand R, Menard D, 2010. Chloroquine clinical failures in *P. falciparum* malaria are associated with mutant Pfmdr-1, not Pfcrt in Madagascar. *PLoS One 5:* e13281.
28. Das MK, Lumb V, Mittra P, Singh SS, Dash AP, Sharma YD, 2010. High chloroquine treatment failure rates and predominance of mutant genotypes associated with chloroquine and antifolate resistance among falciparum malaria patients from the island of Car Nicobar, India. *J Antimicrob Chemother 65:* 1258–1261.
29. World Health Organization, 1996. *Assessment of Therapeutic Efficacy of Antimalarial Drugs for Uncomplicated Falciparum Malaria in Areas with Intense Transmission. WHO Document No. WHO/MAL/96.1077*. Geneva: World Health Organization.
30. Jovel IT, Mejia RE, Banegas E, Piedade R, Alger J, Fontecha G, Ferrreira PE, Veiga MI, Enamorado IG, Bjorkman A, Ursing J, 2011. Drug resistance associated genetic polymorphisms in *Plasmodium falciparum* and *Plasmodium vivax* collected in Honduras, Central America. *Malar J 10:* 376.
31. WHO, 2010. *Global Report on Antimalarial Drug Efficacy and Drug Resistance: 2000–2010*. Geneva: World Health Organization.
32. Neuberger A, Zhong K, Kain KC, Schwartz E, 2012. Lack of evidence for chloroquine-resistant *Plasmodium falciparum* Malaria, Leogane, Haiti. *Emerg Infect Dis 18:* 1487–1489.